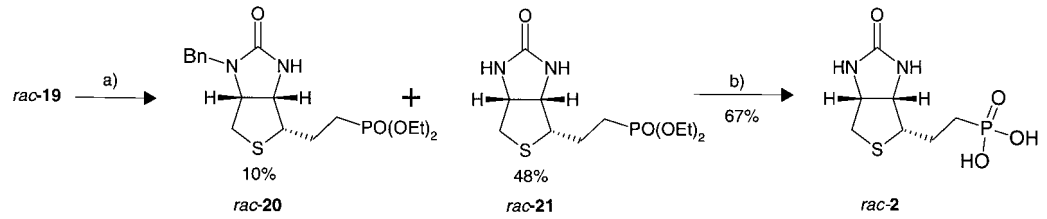

Figure 6. Deprotection. (a): conc.(96%) H_2SO_4, 20°, 16 h; (b):(1) TMSBr (15 equiv.) DCM, 20°, 2.5 days, (2) H_2O.

3 BIOLOGICAL ACTIVITY

Tested at 250 μM **rac-2** and **rac-21** were inactive as inhibitors of adenosylsuccinate synthetase (AdSS).[1]

Both compounds were also inactive when tested as herbicides against a selection of mono- and dicotyledonous weed species at 2 kg ha^{-1} in the Novartis 1a standard screen.

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Plant cell cultures as a model for the biochemistry of crop selectivity

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Abstract: Plant cell cultures have been used to study the metabolic degradation of 4-amino-5-methyl-2-(*tert*-butylaminocarbonyl)-1,2,4-triazolin-3-one. The biochemical basis of selectivity was shown to reside in effective metabolic conjugation. The herbicide was eliminated from cell cultures of beet (which is tolerant to it) by conjugation (*N*-glycolisation), but this occured to only a limited extent with cell cultures of non-target plants such as soybean.

Keywords: crop selectivity; cell cultures; herbicide metabolism

Plant cell suspension cultures are well suited for the study of the metabolic degradation of xenobiotics.[1–3] Here, studies on 4-amino-5-methyl-2-(*tert*-butylaminocarbonyl)-1,2,4-triazolin-3-one (**1**; see Fig 1), which is selective in sugar beet and shows broad-spectrum weed control against monocotyledenous and dicotyledenous species, using plant cell cultures, are presented. The aim of the studies was (i) to establish the pathway of metabolization in target (red beet) and non-target (soybean) plant cell suspension cultures, (ii) to investigate the biochemical basis for tolerance in the target cell culture and for selectivity between target and non-target cell cultures, and (iii) to compare the in-vitro results with the field situation.

Either dark-grown heterotrophic suspension cultures of soybean (*Glycine max* Merr cv *Merill* v *Mandarin*) or light-grown heterotrophic suspension cultures of red beet (*Beta vulgaris conditiva* Alef) were grown in Gamborg B5 medium containing sucrose, (20 g litre^{-1}), and 2,4-D, (0.1 mg litre^{-1}). The ^{14}C-radiolabelled compound was applied in acetonitrile solution. Metabolites were isolated from either the nutrient medium or the cell extract (acetonitrile + water, 8 + 2 by volume), both after partitioning against

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ethyl acetate, by micropreparative HPLC, preparative TLC and AMD-TLC. Identification was accomplished by electron impact and FAB mass spectroscopy and [^{1}H]NMR spectroscopy.

From growth curves with both cultures, and determination of fresh/dry weight, pH value and conductivity, it was evaluated that the active ingredient (AI) had to be applied approximately seven days after culture transfer into fresh medium (late proportional growth phase). The AI had hardly any phytotoxic effect (up to a final concentration of 100 μM [7 μCi]), explained by its mode of action as an inhibitor of photosystem II which is not established in the two heterotrophic cell cultures used.

After a short-time incubation experiment (three days) with red beet and soybean cultures, almost half of the radioactivity was recovered from the cells of the red beet culture, three-quarters of this cell radioactivity being recovered in the polar (aqueous) cell extract fraction. In soybean cells, approximately one-quarter of the cell radioactivity appeared in this fraction. The ratio of radioactivity in the aqueous and organic phases from the respective cells was exactly reciprocal regarding red beet and soybean cells (3:1 against 1:3, respectively). The balance of components in the respective two fractions demonstrated that 95% of the total soybean radioactivity remained as the parent compound . This value was only *c* 65% with the red beet culture, in which a polar component amounted for >25% of the activity. This compound was not detected in the soybean culture.

A kinetic investigation of the behaviour of the parent compound in the two cell cultures revealed significant differences. In the red beet culture, after a four day lag phase, a sharp increase of uptake of the parent compound into the cells was accompanied by a concomitant decrease in the nutrient medium. No such sharp induction was observed with the soybean culture. This situation was matched by a complete change of radioactivity pattern from the organic to the aqueous phase in the red beet cells and later in the red beet medium (pointing to secretion of polar metabolites from the cells into the medium). No such clear induction was observed with the soybean culture: the rates of changes in the cells were very low, and they were almost zero in the nutrient medium. Analysing the phases for their components, the situation described for the phases exactly matched the almost complete migration of radioactivity from the parent into a major polar component and further more polar components in red beet cells, whereas no such change was observed in the soybean culture. There, only a slow decline of the parent and a minor increase in radioactivity in polar materials was observed.

In a scaled-up experiment used for the isolation of metabolites and for the elucidation of the metabolic pathway, red beet and soybean cultures were incubated for 11 and 10 days, respectively, in the presence of 100 μM AI (7 μCi). Both cultures incorporated much more radioactivity into the cells (and into the aqueous cell phases) as compared to the short-duration experiments. In addition, the distribution of radioactivity between the extracts was shifted markedly towards the aqueous phases. Nevertheless, again the remarkable difference between the red beet and soybean cultures regarding polar metabolites was observed: with the red beet culture, in both the nutrient medium and cell extract more than 93% of the radioactivity was recovered in aqueous phases, while these amounted to only 53% (cell extract) and 16% (nutrient medium) with the soybean culture. Analysing the phases for their components, the situation described for the phases exactly matched the complete disappearance of the parent in the red beet culture after 11 days, whereas in the soybean culture still more than 75% remained after 10 days as unchanged parent compound. The main red beet polar metabolite was the parent-*N*-glucoside which was present in the aqueous cell extracts, as well as in the aqueous phase of the nutrient medium. It amounted to 76% of the recovered radioactivity. Most of this compound was extracted from the red beet cells, and a small part, which may have been secreted or generated by extracellular enzymes or by cell lysis, was found in the nutrient medium. A further 22.5% of the radioactivity was detected as more polar metabolites than the parent-*N*-glucoside. In the soybean culture, only *c* 23% of the parent had been metabolized within 10 days, and the parent-*N*-glucoside amounted to only 9% of the recovered radioactivity. The amount of radioactivity in the more polar metabolites was reduced as well (*c* 9%). In total, the polar metabolites amounted to 98.5% of the recovered radioactivity from the red beet culture versus 17.4% from the soybean culture (Table 1).

Besides the parent-*N*-glucoside (76.0%), three further conjugate metabolites were isolated and identified from the red beet culture: parent-*N*-glucoside-2′-*O*-sulfate (12.2%), parent-*N*-glucoside-2′-*O*-glucuronide (5.2%) and a parent-*N*-glycoside (5.1%) whose carbohydrate moiety was unlike glucose. These three complex conjugates were not detected in the soybean culture. From the latter, besides the parent-*N*-glucoside (9.6%), one further conjugate metabolite was partially identified as parent-*N*-glucoside-6′-OR (7.8%). This complex conjugate was not present in the red beet culture (Table 1).

Using the identified metabolites, a metabolic pathway of the title compound in red beet and soybean cell suspension cultures was proposed (Fig 1). Metabolic degradation resulted in 100% transformation into five metabolites in the target red beet culture whereas, besides the parent-*N*-glucoside, only one was present in the soybean culture. In the non-target soybean culture, more than 75% of the parent compound survived. Metabolic degradation resulted in the conversion into four metabolites (plus two very minor metabolites) which together amounted to 21.6%, whereas besides the parent-*N*-glucoside all but one were absent from the red beet culture. The parent-*N*-

Component	*Number*[b]	*Cell culture*	
		Red beet	*soybean*
		% of Total radioactivity[c]	
Parent compound	1	nd[e]	77.4
Parent-*N*-glucoside[d]	2	76.0	9.6
Parent-*N*-glucoside-2′-*O*-sulfate	3	12.2	nd
Parent-*N*-glucoside-6′-OR	4	nd	7.8
Parent-*N*-glucoside-2′-*O*-glucuronide	5	5.2	nd
Parent-*N*-glycoside (not glucose)	6	5.1	nd
Descarbamoyl-parent	7	nd	2.5
Desamino-parent	8	1.5	1.7
Unknown 1		nd	0.8
Unknown 2		nd	0.1
Total		100.0	100.0

[a] Incubated with the parent compound [100 μM] for 10 and 11 days, respectively.
[b] See Fig 1 for structures.
[c] Non-extractable residues (solids; <2%) were not considered.
[d] This was also the main metabolite in sugar beet plants.
[e] nd = not detected.

Table 1. Distribution of parent compound and metabolites in red beet and soybean cell suspension cultures[a]

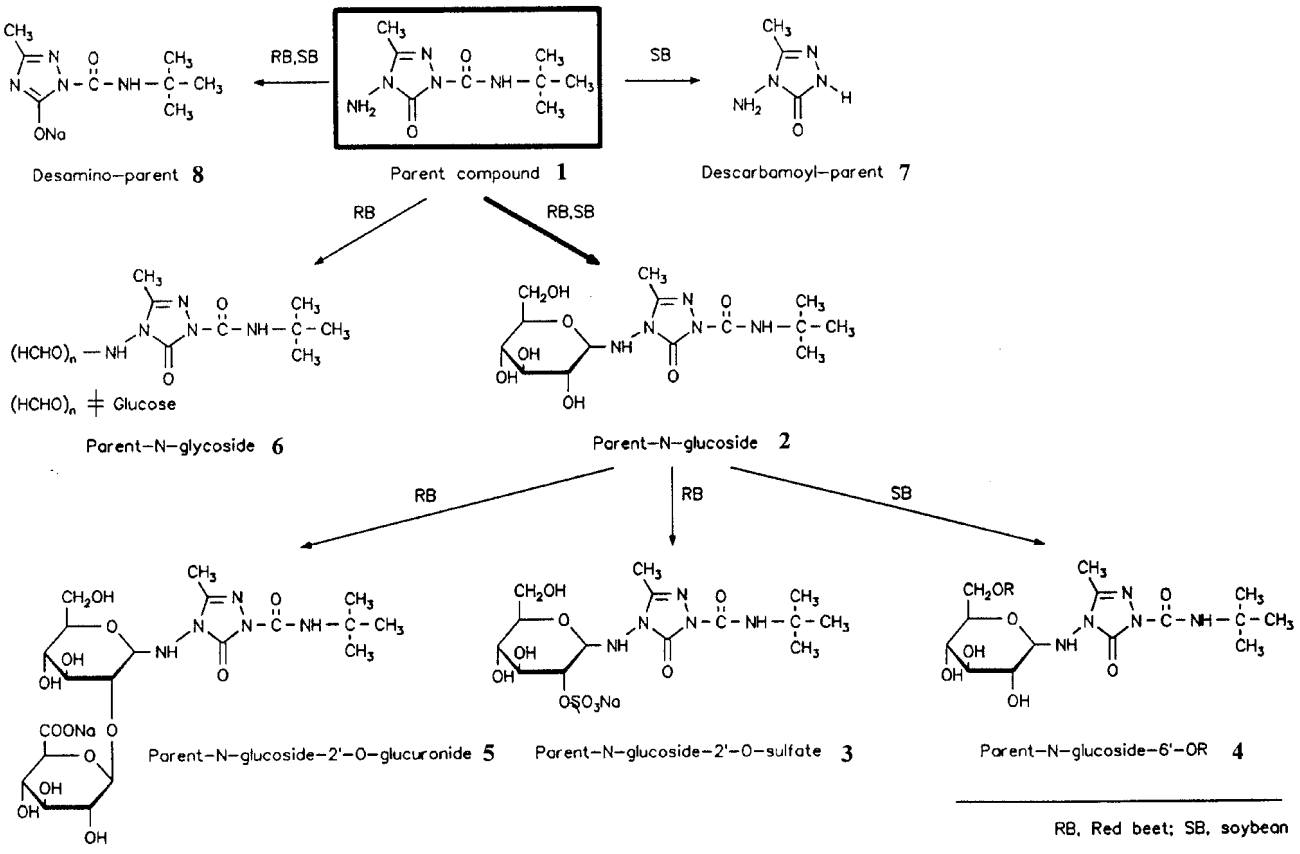

Figure 1. Proposed metabolic pathway of compound **I** in red beet and soybean cell suspension cultures.

glucoside represented the main metabolite in both cultures, although at very different levels (76% in the red beet versus 9.6% in the soybean culture).

In summary:

1. The herbicide was eliminated in beet cell cultures, as in beet crop plants, by conjugation (*N*-glucosylation) (Reiner H – pers comm).
2. In plants, as in cell cultures, the biochemical basis for selectivity was shown to reside in effective metabolic conjugation. With cell cultures of non-target plants, like soybean, elimination by conjugation occurred only to a limited extent.
3. The aglycone of the complex conjugate metabolites was identical in different cultures.
4. The formation of more complex metabolic conjugates varied between cultures.
5. Very rare structures for plant metabolites, like a

2′-*O*-sulfated glycoside and a 2′-*O*-linked glucuronic acid containing disaccharide, were detected in the red beet culture.

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Effect of halomethyl-1,3,5-triazines on nitrification of ammonia

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Abstract: Nitrification inhibitory activity of halomethyl-1,3,5-triazines was determined by measuring the inhibitory activities on ammonia-oxidation to nitrate (NO_3^--N) in an upland soil and on ammonia-oxidation to nitrite (NO_2^--N) by *Nitrosomonas europaea* ATCC 25978 (ATCC) and *Nitrosomonas sp* TK 794 (TK).

Within the chlorinated trimethyl-1,3,5-triazines, those bearing at least one trichloromethyl group inhibited nitrification more strongly, both in soil and in cell suspension of ATCC, than other mono- or dichlorinated methyl-1,3,5-triazines. Introduction of an amino group to 2,4,6-tris(trichloromethyl)-1,3,5-triazine gave 10- and 100-fold increases of nitrification inhibitory activity in soil and ATCC cell culture, respectively. Within the trihalomethyl-1,3,5-triazines, those having tribromomethyl group(s) exhibited rather weaker nitrification inhibition in soil than the corresponding trichloromethyl-1,3,5-triazines, although they showed a strong inhibition in cell suspension.

Ammonium oxidation in ATCC was inhibited more strongly than that in TK. In QSAR studies, the optimum log *P* values were calculated as *c* 4.30. By using this value it will become possible to design highly active trichloromethyl-1,3,5-triazine nitrification inhibitors.

Keywords: halomethyl-1,3,5-triazines; trichloromethyl-1,3,5-triazines; nitrification inhibitors; *Nitrosomonas europaea* ATCC 25978; *Nitrosomonas sp* TK 794; quantitative structure–activity relationship

1 INTRODUCTION

It is known that trichloromethyl-1,3,5-triazines are highly active nitrification inhibitors in upland soil, possibly by controlling ammonia-oxidizing, but not nitrite-oxidizing, bacteria.[1–4] In a quantitative structure–activity relationship (QSAR) study, the hydrophobic parameter, log *P*, was found to be an important parameter affecting inhibitory activity by these 1,3,5-triazines.[3] A mode-of-action study of these inhibitors for ammonia-oxidizing bacteria has been presented elsewhere.[5] In this summary, the nitrification-inhibiting activity of halomethyl-1,3,5-triazines, including trichloromethyl-1,3,5-triazines, was assayed in soil and in cell cultures of ammonia-oxidizing bacteria.

2 MATERIALS AND METHODS

Most of the halomethyl-1,3,5-triazines were synthesized by (co-)trimerization of nitriles, condensation of *N*-(haloacetimidoyl)haloacetamidine with acid anhydride or halogenation of methyl-1,3,5-triazines.[1,6,7] 2-Amino-4-halomethyl-1,3,5-triazines were prepared by nucleophilic amination reaction of corresponding 2-trichloromethyl-4-halomethyl-1,3,5-triazines.[1–3] Nitrification inhibitory activity of the 1,3,5-triazines was determined through measurement of the inhibitory activities on ammonia-oxidation to nitrate (NO_3^--N) in an upland soil and on ammonia-oxidation to nitrite (NO_2^--N) by *Nitrosomonas europaea* Winogr. ATCC 25978 (ATCC) and *Nitrosomonas sp* TK 794 (TK). The nitrification inhibitory indices in soil, cell culture of ATCC and TK were presented as pI_{50} (soil), pI_{50} (ATCC) and pI_{50} (TK), which indicated the logarithm of the reciprocal molar concentration for 50% nitrification inhibition by the compound tested.

3 RESULTS AND DISCUSSION

Within the chlorinated trimethyl-1,3,5-triazines, those bearing at least one trichloromethyl group inhibited nitrification more strongly, both in soil and in cell suspension of ATCC, than other mono- or dichlorinated methyl-1,3,5-triazines. This fact indicated that the trichloromethyl group(s) may be essential for high activity in 1,3,5-triazine nitrification inhibitors. Introduction of an amino group to the 2,4,6-tris(trichloromethyl)-1,3,5-triazine resulted in 10- and 100-fold

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